Managing PAD with multiple platelet inhibitors: The effect of combination therapy on bleeding time

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Lower-extremity peripheral arterial occlusive disease (PAD) identifies patients at high cardiovascular risk and is an indicator of large-burden atherosclerosis. Platelet-inhibiting medications have been shown to significantly reduce cardiovascular morbidity and mortality in high-risk patients.

Oral platelet inhibitors affect platelet function through different mechanisms. Aspirin inhibits cyclooxygenase, thereby reducing thromboxane A_2 production. The ADP receptor on the platelet membrane is blocked by the thienopyridines (ticlopodine and clopidogrel), and platelet cyclic AMP phosphodiesterase is inhibited by cilostazol and dipyridamole. The Antiplatelet Trialists' Collaboration has summarized the benefits of platelet inhibitors in patients at high cardiovascular risk[1,2] These studies have shown a reduction in major ischemic events and death, and as a result, aspirin has become a mainstay in the management of these patients. More recently, the thienopyridine clopidogrel was compared with aspirin in the CAPRIE (*C*lopidogrel versus *A*spirin in *P*atients at *R*isk of *I*schemic *E*vents) trial, a large prospective, randomized study. The results of CAPRIE demonstrated an 8.7% risk reduction of major ischemic events in the clopidogrel-treated group compared to aspirin.[3] Subgroup analysis of the CAPRIE trial demonstrated a greater relative risk reduction (23.9%) of ischemic events in patients with PAD. The CAPRIE results established the benefit of clopidogrel in patients with peripheral arterial disease. The short- and long-term benefit of combined platelet inhibition with aspirin and clopidogrel in patients with cardiovascular diseases has recently been documented in the CURE (*C*lopidogrel in *U*nstable Angina to Prevent *R*ecurrent *E*vents) trial.[4,5]

The most common symptom of patients with PAD is intermittent claudication. Cilostazol has been shown to significantly improve walking distance and quality of life in patients with intermittent claudication.[6-8] Patients requiring treatment with cilostazol may be simultaneously receiving other platelet inhibitors. This raises the issue of enhanced platelet inhibition by the combined presence of multiple platelet inhibitors. Because multiple pathways of platelet function are altered, there is a potential that additive or synergistic platelet inhibition may occur.

The template bleeding time is an in vivo measure of overall platelet hemostatic function and can be used to assess the effect of platelet inhibition.[9] Platelet-inhibiting drugs used for treatment of arterial disease prolong the bleeding time. Determination of the effect on the bleeding time remains a major component of the evaluation of any new platelet-inhibiting drug. Despite a lack of evidence correlating preoperative bleeding time to clinical bleeding,[10-12] this measurement provides valuable information regarding the primary hemostasis mediated by platelets and their interaction with the biologically active vessel wall. In order to address the effect of platelet inhibitors, both singly and in combination, in patients with peripheral arterial disease, a prospective study was performed using the three most commonly indicated medications in this patient population.

MATERIAL AND METHODS

Between June 2000 and March 2002, 26 patients were evaluated for enrollment in this prospective study, and 21 were entered. The mean age of the patients was 65.9 ± 8.43 years with 71.4% males and 48% smokers. Twelve (57%) were Caucasian and 43% African-American. Seven patients (33.3%) were found to have diabetes insipidus. Eligibility criteria included the presence of PAD causing intermittent claudication, an ABI of less than 0.90, and no contraindications to use of the study medications. The subjects were required to have normal liver enzymes, prothrombin, and partial thromboplastin times, and no history of congestive heart failure or renal insufficiency.

The severity of lower-extremity occlusive disease was classified by using the clinical categories of chronic limb ischemia, as suggested by Rutherford et al.[13] All patients except one (n = 25) in this study were classified as grade I, which is characterized by the complaint of claudication. One patient was grade II, defined as suffering from ischemic rest pain. Nine patients underwent further categorization by treadmill testing. Six had results classifying them as

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Competition of interest: none

Presented at the Thirty-first Annual Meeting of the Society for Clinical Vascular Surgery, Miami, Fla, Mar 4-9, 2003.

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J Vasc Surg 2003;38:710-13.

0741-5214/2003/$30.00 + 0

doi:10.1016/S0741-5214(03)01029-2

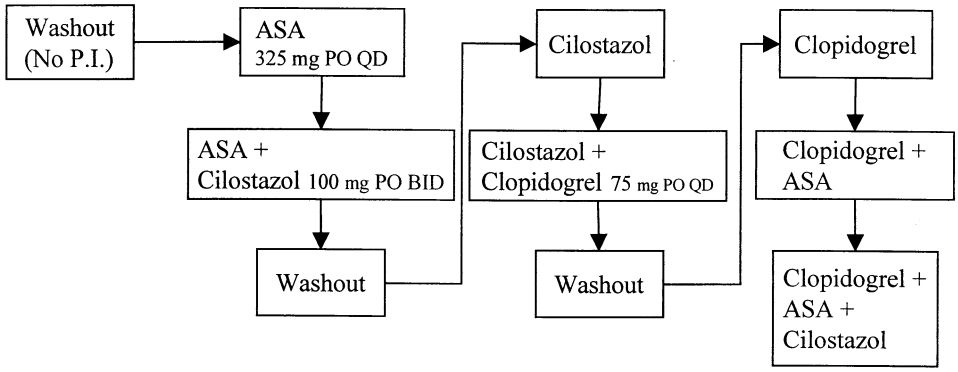

Fig 1. Outline of treatment program. Each treatment phase lasted for 14 days, with an initial washout period, during which the patient took no platelet-inhibiting medications. Washout was repeated throughout the protocol as necessary in order to prevent carryover of drug effects.

category 2, two were category 1, and one patient was category 3.

Five patients were found to be ineligible and were dismissed after an initial screening visit. Reasons for ineligibility included a lack of available venous access for repeated blood sampling (n = 2), inability to comply with the prolonged study period (n = 2), and inability to discontinue current platelet inhibition (n = 1). One patient left the study during the third visit, due to palpitations that developed shortly after commencing cilostazol therapy.

Each patient underwent treatment with each possible combination of aspirin (325 mg daily), clopidogrel (75 mg daily), and cilostazol (100 mg twice a day). Initially, patients underwent a 14-day "washout" period, during which all platelet inhibitors were discontinued. Patients then underwent sequential treatment with every possible combination of these medications. Each phase was 14 days in duration. Washout periods were used during the treatment algorithm in order to avoid any carryover of the effect of various treatments. The entire treatment program is diagrammed in Fig 1. This study was evaluated and approved by the Institutional Review Board of the Temple University Hospital, and all patients signed an approved informed consent.

At the end of each treatment phase, patients were examined by a physician. The bleeding time was measured by using a standardized protocol. The bleeding times were performed by 3 trained technicians, each of whom had performed the test with acceptable results in at least 10 normal persons prior to beginning studies in the patients. These technicians were not blinded to the study design or to the patient treatment schedule. Under this protocol, venous hypertension was established with a 40 mm Hg venous tourniquet. A Surgicutt (International Technidyne Corporation, Edison, NJ) automated scalpel was used to make a 5-mm-long, 1-mm-deep horizontal incision located 1 cm below the antecubital crease. Filter paper (ITC) was used to dab away excess blood every 30 seconds until no blood was collected by the paper; at which time, the examination was deemed complete. A maximum of 20 minutes was allowed for bleeding time determination. If the patient continued to bleed at 20 minutes, the cuff was deflated, pressure was applied, and the bleeding time was recorded as 20 minutes.

STATISTICAL METHODS

The dependent variable bleeding time was treated as a continuous variable for all analyses. Means, standard deviations, and number of observations were presented for each variable. The experimental unit was each individual subject (n = 21). The experimental design was a single factor repeated measures design. Prior to analysis, all data were tested for normality by using the Shapiro-Wilk test. The data were significantly non-normal for all variables. In order to apply analysis of variance (ANOVA) methods, a "normalized-rank" transformation was applied to the data. The rank-transformed data was analyzed by using a mixed-model ANOVA for repeated measures followed by multiple comparisons to detect significant mean differences between treatments. Multiple pair-wise comparisons used the Dunn-Bonferroni adjustment to maintain an experiment-wise type I error of 0.05 or less. Differences between means were considered significant if the probability of chance occurrence was <0.05 using two-tailed tests.

RESULTS

The results of bleeding time measurements are presented in Fig 2. The average baseline bleeding time for the group was 4.29 minutes (SD, 1.69). Treatment with cilostazol alone did not produce a significant change in bleeding time (BT = 5.41 ± 2.69 minutes). Individual treatment with either aspirin (BT = 6.64 ± 3.52, $P < .01$) or clopidogrel (BT = 10.17 ± 5.4, $P < .01$) caused a significant increase in bleeding time over the baseline.

The effects of combination therapy varied among medications. When cilostazol was added to either aspirin (ASA + Cilo BT = 8.3 ± 4.27) or clopidogrel (Clop + Cilo BT = 12.7 ± 7.46), no significant additional effect on bleeding

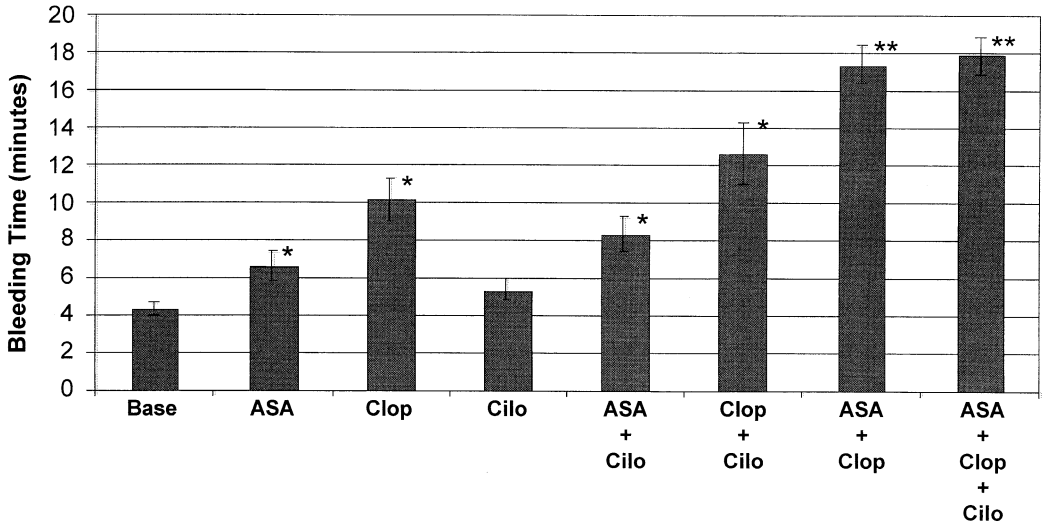

Fig 2. Average bleeding time for each treatment phase. Error bars demonstrate SE. *Base*, Baseline BT; *ASA*, aspirin 325 mg QD; *Clop*, clopidogrel 75 mg QD; *Cilo*, cilostazol, 100 mg BID. *$P \leq .05$ versus baseline. **$P \leq .05$ versus all single agents and versus ASA + Cilo and Clop + Cilo.

time was noted. The combination of aspirin and clopidogrel (BT = 17.39 ± 4.59) resulted in an increase in bleeding time that was significant when compared with treatment with either drug alone.

Triple therapy demonstrated a significant increase in bleeding time when compared with the cilostazol-based dual drug combinations (ASA + Clop + Cilo BT = 17.92 ± 4.69, $P < .01$ vs. ASA + Cilo and Clop + Cilo) as well as with each drug alone. When compared with aspirin plus clopidogrel, the addition of cilostazol did not affect the bleeding time.

DISCUSSION

With increasing understanding of cardiovascular risk, and clinical experience with platelet inhibitors, it becomes apparent that combined platelet inhibition with aspirin and clopidogrel may be indicated for patients with PAD. This subgroup of high-risk patients has been shown to significantly benefit from clopidogrel compared with aspirin alone.[3] In the subset of PAD patients who have suffered acute coronary ischemia, prolonged platelet inhibition with aspirin and clopidogrel has been shown to be beneficial.[4,5] In addition, prospective blinded trials are now underway to compare combined platelet inhibition with monotherapy in patients with PAD.

Our data demonstrate that aspirin has a significant platelet-inhibiting effect compared with baseline values and that clopidogrel has a greater effect than aspirin. The combination of aspirin and clopidogrel significantly prolongs bleeding time compared with either agent alone. A major feature of this study is that these measurements were performed in the same patients over the course of a prospective treatment scheme, and each subject served as his or her own control.

Aspirin and clopidogrel have previously demonstrated a synergistic effect when used in combination. In an ex vivo model, Moshfegh et al[14] demonstrated that combining aspirin and clopidogrel provided a significantly greater inhibition of ADP-mediated platelet aggregation than either drug alone. Studies in animals have demonstrated that combined platelet inhibition with aspirin and clopidogrel is more effective than are single agents in reducing thrombus weight in an ex vivo model of stent thrombosis.[15,16]

Many patients with PAD have symptoms of intermittent claudication and require treatment with cilostazol. The bleeding time after treatment with cilostazol alone is not significantly different from the baseline. Cilostazol added to either aspirin or clopidogrel does not alter the bleeding time compared with either agent alone. Furthermore, when cilostazol is added to the combination of aspirin and clopidogrel, no additional effect is observed.

Although this is a small trial, it is focused on the effect of oral platelet inhibitors on the bleeding time in patients with lower-extremity PAD. The sequence of administration of study drugs was specifically designed so that triple-drug therapy was the final study segment. As such, the study drugs were not randomized, which could add potential bias to our observations, although we believe that this is unlikely.

Numerous laboratory tests have been designed to evaluate various aspects of platelet function. These include assessment of platelet function in vitro by using platelet aggregometry, the platelet function analyzer (PFA-100), and other methods to specifically evaluate platelet responses, such as platelet secretion or signaling events. We chose the bleeding time as the end point for this study as it reflects a readily available clinical measure of platelet hemostatic function and has been extensively used to evaluate the

effect of all platelet-inhibiting drugs. Although prior studies have failed to specifically link bleeding time results to perioperative bleeding complications,[10-12] the number of confounding variables associated with such observations probably masks the true association. The CURE investigators reported more major bleeding complications with combined platelet inhibition using aspirin plus clopidogrel than with aspirin alone.[4] As observed here, the combination of aspirin plus clopidogrel provided a significantly greater bleeding time than did either of these agents alone, any other single agent, or any other dual combination. We believe that there is likely to be a clinically relevant link between our observations of the bleeding time effect of combined platelet inhibitors and the increased bleeding complications observed in the CURE trial.

Our data suggest that cilostazol is a relatively weak platelet inhibitor compared to aspirin and clopidogrel. Combining cilostazol with aspirin or clopidogrel does not alter the bleeding time compared with either agent alone. Although the combination of aspirin and clopidogrel has a significant added effect on bleeding time compared with either agent alone, the addition of cilostazol to this combination does not appear to alter platelet function. These results suggest that when combined platelet inhibition with aspirin and clopidogrel is indicated because of cardiovascular risk, bleeding time is prolonged, which is consistent with the clinical results noted in randomized trials. However, when cilostazol is required because of symptoms of intermittent claudication, it can be added without additional perturbation in platelet function as reflected by the bleeding time.

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Submitted May 1, 2003; accepted Jun 26, 2003.